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Molecular simulation as an aid to experimentalists

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Computer-based molecular simulation techniques are increasingly used to interpret experimental data on biomolecular systems at an atomic level. Direct comparison between experiment and simulation is, however, seldom straightforward. The available experimental data are limited in scope and generally correspond to averages over both time and space. A critical analysis of the various factors that may influence the apparent degree of agreement between the results of simulations and experimentally measured quantities is presented and illustrated using examples from recent literature.

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Introduction

Computer-based molecular simulation techniques have become a standard tool to facilitate the interpretation of experimental data at an atomic or molecular level. If the assumptions and approximations inherent in a simulation model, such as (1) which degrees of freedom are simulated, (2) how the forces acting on the particles are approximated, (3) how the system is propagated in time and (4) the nature of the thermodynamic and spatial boundary conditions, are appropriate [1], any property $Q(\mathbf{r}, \mathbf{p})$ that depends primarily on the coordinates \mathbf{r} and momenta \mathbf{p} of the chosen degrees of freedom can in principle be calculated and compared to its experimental counterpart. Experimental properties commonly compared to atomistic simulations include X-ray or neutron diffraction intensities, NMR parameters (i.e. chemical shifts, J-coupling constants, relaxation times and dipolar-couplings), various quantities derived from EPR, CD,

infrared, Raman or fluorescence spectroscopy, data derived from measurements of fluorescence energy transfer, and even electrical or mechanical response functions (e.g. AFM).

There are two basic reasons why molecular simulations can facilitate the interpretation of experimental data from biomolecular systems. The first is that experimentally measured properties correspond in general to averages of a quantity Q over both space and time, $\langle Q \rangle_{\text{space,time}}$ where $\langle Q \rangle$ is an average over the distribution $P(Q(\mathbf{r}, \mathbf{p}))$ of Q -values depending on single configuration and momentum values [2]. The averaging inherent in the measurement means it is usually not possible to determine the Q -distribution $P(Q(\mathbf{r}))$ or the configurational distribution $P(\mathbf{r})$ from $\langle Q(\mathbf{r}) \rangle_{\text{exp}}$ (note, the \mathbf{p} -dependence has been omitted for simplicity). Furthermore, representing the conformational distribution $P(\mathbf{r})$ by one conformation derived from, for example, X-ray crystallography, may lead to inconsistencies when calculating alternative experimental quantities that have a different dependence on $P(\mathbf{r})$. Examples of this situation can be found in references [3–5]. Measured NOE intensities may result from different molecular conformations [3]. Seemingly contradictory data from X-ray and NMR studies of the *cis-trans* interconversion induced by cyclophilin could be explained from the conformational distribution obtained by MD simulation [4]. Small-angle X-ray scattering profiles calculated from simulated ensemble averages agree better with experimental data than profiles calculated from single X-ray crystal structures [5]. The second reason simulations facilitate the interpretation of experimental data is that the number of quantities N_Q^{exp} that can be measured for a molecular system is generally very much smaller than the number of degrees of freedom N_{dof} of the system. This makes the problem of determining the conformational distribution $P(\mathbf{r})$ from the set of $\langle Q(\mathbf{r}) \rangle_{\text{exp}}$ values highly underdetermined [6–8] even if combining alternative sets of experimental data pertaining to one system could improve the situation [9].

These problems can be circumvented partly by using molecular simulations to generate conformational distributions $P(\mathbf{r})$ from which averages $\langle Q(P(\mathbf{r})) \rangle$ can be obtained and compared or coupled to experimental values $\langle Q \rangle_{\text{exp}}$ [10]. The use of molecular simulations to interpret experimental data is growing rapidly in popularity with progressively more indirect and adventurous comparisons being made. In light of this we feel it is appropriate to focus this contribution on the potential pitfalls of using molecular simulation in this manner. In particular, we present simple criteria against which such studies can be

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judged and illustrate these with reference to recent literature.

Interpretation of experimental data using simulation

When relating the *average* of a property over a given conformational distribution $P(\mathbf{r})$, whether from a simulation ($\langle Q \rangle_{\text{sim}}$) or measured experimentally ($\langle Q \rangle_{\text{exp}}$), to the conformational distribution itself, three general cases can be distinguished:

- (Q1) $\langle Q \rangle$ does not reflect the shape of $P(\mathbf{r})$ as $\langle Q \rangle$ is insensitive to conformation; see, for example, reference [11].
- (Q2) $\langle Q \rangle$ does not reflect the shape of $P(\mathbf{r})$ as $\langle Q \rangle$ is determined by rarely sampled conformations with small (*irrelevant*) Boltzmann weights; see, for example, reference [12].
- (Q3) $\langle Q \rangle$ reflects the dominant conformations of $P(\mathbf{r})$.

Only in the case Q3 can $\langle Q \rangle_{\text{sim}}$ carry information relevant to the interpretation of $\langle Q \rangle_{\text{exp}}$ at a molecular level. Nevertheless, agreement between $\langle Q \rangle_{\text{sim}}$ with $\langle Q \rangle_{\text{exp}}$ may be obtained if:

- (A1) $\langle Q \rangle$ is insensitive to $P(\mathbf{r})$, that is, $\langle Q \rangle_{\text{sim}}$ matches $\langle Q \rangle_{\text{exp}}$ irrespective of the $P(\mathbf{r})$ being simulated [1].
- (A2) There are compensating errors in the simulation model, procedure or experimental set-up [13]; see D1-5 below.
- (A3) The experimental data of interest, $\langle Q \rangle_{\text{exp}}$, have been used to bias the simulation.
- (A4) $\langle Q(\mathbf{r}) \rangle_{\text{sim}}$ is sensitive to the distribution $P(\mathbf{r})$.

Again, only in the case A4 can the degree of agreement between $\langle Q \rangle_{\text{sim}}$ and $\langle Q \rangle_{\text{exp}}$ be used to validate the simulation and/or to interpret the experimental results.

Failure to observe a correlation between the simulation and experiment can also be due to many reasons:

- (D1) The simulation is insufficiently accurate, that is, (a) relevant degrees of freedom were omitted; (b) the force field was insufficiently accurate; (c) approximations made when solving the equations of motion were too crude; (d) inappropriate thermodynamic or spatial boundary conditions were used.
- (D2) The measured $\langle Q \rangle_{\text{exp}}$ is inaccurate.
- (D3) $\langle Q \rangle_{\text{sim}}$ and $\langle Q \rangle_{\text{exp}}$ are averaged differently with respect to time or spatial extent.
- (D4) Related but different quantities are compared, for example, atom-positional fluctuations versus crystallographic B factors.
- (D5) Different systems are compared (e.g. crystal versus solution), or systems studied under different

thermodynamic conditions (e.g. temperature, pressure, pH, ionic strength, etc.).

Clearly the interpretation of experimental observables in terms of molecular conformations is not straightforward. In particular, the degree of agreement between $\langle Q \rangle_{\text{sim}}$ and $\langle Q \rangle_{\text{exp}}$ can easily be over interpreted and frequently leads researchers to make unrealistic claims regarding the validity of a simulation (when an apparent correlation between theory and experiment is observed) or, equally problematic, leads to the unjustified disregard of simulation results (when no correlation is evident).

Note, in all of the above $\langle Q \rangle_{\text{exp}}$ denotes an observable quantity, that is, a property that can be measured directly. Such primary experimental data should not be confused with secondary experimental data, Q' , that is, data derived from $\langle Q \rangle_{\text{exp}}$ by applying a given procedure, f , based on various assumptions and approximations: $Q' = f(\langle Q \rangle_{\text{exp}})$. For example, whereas peak location and intensity from X-ray diffraction or NMR spectroscopic experiments represent primary data, molecular structures, NMR order parameters, and so on are secondary (derived) quantities. Comparisons of simulations to secondary, non-observed, experimental quantities reflect, at least partly, the approximations and assumptions associated with the conversion and may in reality carry little experimental information [6]. Clearly, when coupling (restraining) a simulation to a set of $\langle Q \rangle_{\text{exp}}$ values in order to ensure that the conformational distribution satisfies $\langle Q(\mathbf{r}) \rangle_{\text{sim}} = \langle Q \rangle_{\text{exp}}$, only primary experimental data should be used. For further discussion on the validation of molecular simulations see reference [14].

Experimentally observable quantities

Below, we illustrate the use of biomolecular simulations to interpret a range of experimentally observed quantities Q in terms of the considerations outlined above using examples from the recent literature. One quantity that is relatively insensitive to the particular conformational distribution is the small-angle X-ray scattering intensity, from which the radius of gyration can be derived. As shown in reference [7] very different conformational distributions can give rise to a given experimental observation. This study also illustrates the difference between comparing to primary data (X-ray intensities) as opposed to secondary data (radius of gyration). Questionable comparisons to secondary quantities are made in many studies. For example in [15] fluorescence measurements on the Trp-cage protein are used to infer changes in enthalpy, which are used to infer changes in the number of hydrogen bonds as a function of temperature, which are ultimately related to results from simulations [15]. In a similar manner the validity of simulations performed with J-coupling constants was assessed by comparing to proposed hydrogen bond energies instead of primary quantities [16]. In such cases the basic comparison is

between alternative modeling procedures, not between simulation and experiment. By contrast, where the comparison is to primary experimental quantities such as infrared spectra [17,18] or NMR spectra [19,20], a direct validation of the simulation models by experiment is possible.

The use of the so-called phi-values to restrain or validate folding simulations deserves a special comment. Phi-value analysis is a useful framework within which to analyze experimental data on folding transitions in proteins [21]. Phi-values are secondary data derived from the effect of mutations on stability and folding kinetics. An equivalent quantity cannot be extracted from simulations; instead phi-values are often defined in terms of the so-called 'native contacts'. Clearly, as the definitions of these two quantities are very different and even span different ranges, any apparent correlation can neither be used to validate the simulation nor to interpret the experiment (see D4 and D5 above) [22–24]. A comparison of simulated versus measured folding rates for proteins [25,26] based on many short simulations is also problematic owing to the different time scales accessible to simulations and experiment (D3), the definition of when the system is folded and assumptions in regard to the probability of folding at long time scales.

In contrast to X-ray or NMR studies that generally yield thousands of individual parameters (diffraction intensities or atom–atom resonances), in some types of experiment only a single quantity is measured, for example, atomic force microscopy, ionic conductance measurements or wide angle X-ray spectra. Such data are generally not suited for model validation owing to inability to relate the measurement to specific degrees of freedom within the model and thus the high probability of compensation of errors (A2 above) [27–30]. This problem is further aggravated when the quantity in question represents secondary experimental data such as in the calculation of the area per lipid, NMR order parameters along the lipid tails in membrane systems or even density profiles across membranes [31,32].

A constant difficulty in the study of biomolecular systems including proteins has been the limited availability of experimental data with high information content. Fortunately, the range of experimental quantities that are being compared to simulations continues to expand. For example, the response of groups of atoms within proteins to local electric fields has been measured experimentally and analyzed using simulations [33]. Comparisons between measured and calculated Raman spectra have provided new insight into the dynamics of water surrounding sugars [34]. Measurements of fluorescence anisotropy [35] and fluorescence lifetimes [36,37] have also been directly compared to the results of simulations

providing novel insights into local dynamics as well as helping to validate the simulation models. MD simulations have shown mobile water molecules inside cavities in proteins that are responsible for detectable NOEs [38] or electron density [39], which could not be traced to fixed water molecules.

Finally, we must draw attention to a few studies [40–43] in which the experimental data $\langle Q \rangle_{\text{exp}}$ that have been used to bias the simulation when generating a conformational distribution $P(\mathbf{r})$ is subsequently used to compare $\langle Q(P(\mathbf{r})) \rangle_{\text{sim}}$ with $\langle Q \rangle_{\text{exp}}$ (see A3 above). Such comparisons do not validate the simulation nor demonstrate that the conformational distribution reflects experiment. In fact, restraining $Q(\mathbf{r})$ in the simulation to match $\langle Q \rangle_{\text{exp}}$ can easily introduce artifacts in $P(\mathbf{r})$ even though the correct average $\langle Q \rangle_{\text{sim}} = \langle Q \rangle_{\text{exp}}$ is maintained, enhancing the likelihood of inappropriate conclusions being drawn [44].

Conclusions

Without question molecular simulations play a crucial role in facilitating the interpretation of experimental data at the atomic level. Nevertheless, it must be remembered that compared to the number of degrees of freedom within biomolecular systems of interest, experimental data are in general very limited in nature. In addition, almost all measured properties are inherently averaged quantities. As a consequence, comparisons between simulations and experiment while seemingly straightforward can be easily overinterpreted or misinterpreted both in terms of the apparent quality of a simulation as well as in terms of the inability of the model to reproduce the experimental observable of interest. In particular, caution must be exercised when comparing simulations to secondary or derived experimental data. The use of secondary data as restraints in simulations often leads to artifacts and should be avoided where possible. In all cases where experimental data are compared with or incorporated into a simulation care must be taken that the data relate to the same molecular system under the same thermodynamic conditions and correspond to the same physical quantities as in the simulation. Finally, experimental data that have been used to bias or restrain a simulation cannot subsequently be used for validation. Failure to observe these simple considerations will make it more likely that excessive claims regarding the validity of simulation results are made or for simulation results to be rejected without justification. More seriously it will mean that inappropriate or even incorrect physical interpretations of experimental data will be propagated in the literature.

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